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product was purified (High Pure PCR Product Purification Kit; Roche Diagnostics Belgium, Brussels, Belgium) and subsequently used for transcription of one specific strand. A mutant T7 RNA polymerase (T7 R&DNA™ polymerase; Epicentre, Madison, WI) with the ability to incorporate both dNTPs and rNTPs was used in the transcription reactions. In addition to a transcription with the regular ribonucleotide substrates, one reaction was performed where CTP was replaced by dCTP, while in two more separate transcriptions either dUTP or dTTP replaced UTP. The transcription reactions were run in a 50 µl volume containing: 40 mM Tris-Ac (pH 8.0), 40 mM KAc, 8 mM spermidine, 5 mM dithiothreitol, 15 mM MgCl<sub>2</sub>, 1 mM of each rNTP, 5 mM of dNTP (in these cases the appropriate NTP was excluded), ~40 nM DNA template (~2 pmol), and 250 units T7 R&DNA™ polymerase. Incubation was performed at 37° C for 2 hours. After transcription, the full-length T7 *in vitro* transcripts (118 nucleotides) were purified by allowing them to anneal to the 5'-biotinylated form of the complementary reverse PCR primer (Figure 5) followed by capture of the biotinylated annealing products onto streptavidin-coated magnetic beads. To this end, 50 pmol biotinylated reverse primer was added to the transcription reactions. The mixtures were first incubated 5 min at 70°C and, subsequently, ~30 min at room temperature. Then, a slight excess of Sera-Mag™ streptavidin magnetic microparticles [Seradyn Inc, Indianapolis, IN; resuspended in 50 µl of 2M NaCl, 20 mM Tris-HCl (pH 8.0), 2 mM EDTA] was added and the resultant mixture incubated at room temperature for 30 min with agitation. A magnetic particle collector (MPC; Dynal, Oslo, Norway) was used to collect the beads, remove the supernatant and, subsequently, to wash the beads three times with 100 µl 100 mM (NH<sub>4</sub>)<sub>3</sub>-citrate. The beads were finally resuspended in 3 µl 25 mM (NH<sub>4</sub>)<sub>3</sub>-citrate containing 0.5 µg bovine pancreas RNase-A (50U/mg; Roche Diagnostics Belgium, Brussels, Belgium) and incubated at room temperature for about 30 min to digest the transcripts to completion. 1 µl of this RNase reaction was removed and added to 5 µl matrix solution. This 1:1 acetonitrile:H<sub>2</sub>O matrix solution is saturated with 3-hydroxypicolinic acid (~100 mg/ml), and further contains 25 mM (NH<sub>4</sub>)<sub>3</sub>-citrate, (occasionally) 2 pmol/µl of an oligonucleotide serving as an internal standard, and cation-exchange beads in (NH<sub>4</sub>)<sup>+</sup>-form (Dowex 50W-X2; Sigma, Saint-Louis, MO) to minimize the presence of sodium and potassium adducts. After incubating the mixture at room temperature for 15 min, 1 µl was put on the sample plate and allowed to dry. Mass spectra were collected using a Reflex III mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany).